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Change in the Number Distribution of Complex Shear Modulus of Single Cells by Actin Cytoskeleton Modifications Measured by Atomic Force Microscopy

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The rheological properties of living cells strongly depend on their cytoskeletal structures, which are composed of polymer networks and responsible for fundamental cellular functions. In particular, the actin network plays a major role in determining the rheological properties of living cells. In order to elucidate how the rheological properties of individual cells are affected by actin filamentous structures, we measured the number distribution of complex shear modulus of single cells, which were treated by actin modification drugs and cultured on microarray substrates, by atomic force microscopy. A force modulation mode experiment was employed to measure the complex shear modulus of single cells in a frequency range of 2-200Hz. When the cells were treated with actin-stabilizing drug, jasplakinolide, and actin-disrupting drug, cytochalasin D (CD), the storage modulus G' and loss modulus G'' increased and decreased, respectively. The changes in G'' were smaller comparing to those in G'. The moduli exhibited a weak power-law dependence on frequency [1], whereas the increasing and decreasing of G' and G'' were accompanied by a decreasing and increasing power-law exponent respectively. Furthermore, their corresponding logarithmic standard deviation σ showed a slight change in the case of jasplakinolide treatment whereas it became small and attained a constant value at higher frequencies in CD treatment [2]. The results indicated that individual differences of cell rheology enhanced as actin cytoskeletal structures were stabilized. Furthermore, it was implied that the observed frequency dependence of σ was attributed to a frequency susceptibility of actin filaments.

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[2] S. Hiratsuka et al., Ultramicroscopy, vol. 109, pp. 937-941, 2009.

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AFM and KPFM Study of Effect of Cholesterol and Cortisol on Structure of Lipid Monolayers

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The plasma membrane is a complex structure, composed primarily of phospholipids and other macromolecules, such as proteins, sterols and steroids, which define its complex functions. Phospholipid monolayers are widely used as convenient models to study plasma membrane. We investigated model lipid monolayers composed of dipalmitoylphosphatidylcholine (DPPC) in order to elucidate effect of cholesterol and cortisol on the structure and properties of these model systems. Supported DPPC monolayers were prepared with Langmuir-Blodgett monolayer technique and imaged by Atomic Force Microscopy (AFM) and Kelvin Probe Force Microscopy (KPFM). Frequency modulation KPFM technique (FM-KPFM) (1) based on electrostatic force gradient was used to measure local surface potential distribution in monolayers. This technique is performed by applying modulation frequency in addition to the resonance frequency of the cantilever and has been shown previously to have superior resolution for measuring surface potential in biomolecular films (2). We found that cholesterol and cortisol produced structural changes (nanoscale domains) both in topography and surface potential maps of the monolayers. In addition with Differential Scanning Calorimetry (DSC) we found that both steroids produce changes the phase transitions of the DPPC membrane.

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The Role of -Galactofuranose in Cell Wall Surface Structure and Elasticity of Aspergillus Nidulans

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The fungal cell wall is a first line of defence from the external environment or chemical treatments. Glucan, chitin and mannan are the main components of *Aspergillus nidulans* hyphal cell wall. β-galactofuranose is a minor component of the cell wall and carries out crosslinking of the other cell wall components, therefore responsible for maintaining cell wall structural integrity. The objective of this study is to reveal the role of β-galactofuranose on structure and physical properties of the hyphal cell wall. Based on its unique capacity to image live samples, atomic force microscopy was used to determine both the ultrastructure and mechanical properties of the hyphal cell wall. Four different knock-out strains of *Aspergillus nidulans* (ugeAΔ, ugeBΔ ugeA/BΔ and ugmAΔ,) that compromise β-galactofuranose synthesis were compared with the wildtype strain (AAE1). Atomic force microscopic imaging and force spectroscopy the mutant wild type strains suggest that a lack of galactofuranose reduces the integrity of cell wall components, where the surface subunits of UgeAΔ and UgmAΔ are 2 times and 4 times larger than that of the wildtype(AAE1) respectively. UgeB shows similar sized subunits as AAE1, in direct contrast to the complete disruption of the structural organization for the cell wall surface of the double mutant ugeA/BΔ. The structural changes are accompanied by a change in viscoelasticity, where the wild type strain is the most elastic. We propose that the lack of galactofuranose disrupts the proper packing of cell wall components, giving rise to more disordered surface subunits and therefore greater pliability.

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Manipulation of Single Knotted DNA Molecules with Topoisomerase II by using Atomic Force Microscopy

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Although the hereditary information is encoded in the primary sequence of the genetic material, much of the regulatory information of DNA is hidden in its topology and geometry in the cell. DNA supercoiling and knotting are important for DNA packaging within all cells, however this impairs the well behavior of multiples cellular processes such as replication and transcription, which involve helical winding, strand separation, and movement along the DNA. The steric and topological problems associated with supercoiling can be studied with numerous different tools among which scanning probe microscopy is a very promising one because of its capability to image on line and with a high resolution, biological samples in nearly physiological conditions.

In this work we studied the way enzymes and drugs interact with DNA molecules by using Atomic Force Microscopy (AFM). Particularly, we have centered our studies on human type II DNA topoisomerases (Top2), which are essential and ubiquitous enzymes that perform important intracellular roles in chromosome condensation and segregation, and in regulating DNA supercoiling. We will present real time movies showing how the Top2 relaxes DNA. In addition we will present AFM images showing the way DNA changes its topology after being exposed to intercalating agents. We hope that this new methodology will unveil some of the still poorly known DNA-proteins and drugs interaction.

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The Mechanical Properties of Electrospun Collagen/PCL Single Fibers and Fibrous Scaffolds

Stephen Baker.

Collagen/Poly-ϵ-caprolactone (PCL) blended fibers were electrospun from a solution of 2.5% wt./vol. bovine collagen, 2.5% wt./vol. PCL and 95% 1,1,1,3,3,3-hexaflouro-2-propanol (HFP). A combined atomic force microscopic (AFM)/optical microscopic technique was used to study the properties of individual fibers in buffer. Mechanical testing of fibers was done using the AFM to laterally stretch individual fibers suspended over 13.5μm wide groves in a transparent substrate. The optical microscope, located below the sample, was used to monitor the stretching process. Fibrous scaffolds of these blends were also electrospun to test the bulk mechanical properties. A total of 5ml of 5% wt./vol. solution of blended collagen/PCL was used to electrospin scaffolds with a 4.75mm inner diameter to be used to for blood vessel tissue replacements. Dots are applied to the surface of the scaffold which is then subjected to cyclic loading under wet conditions while video is being recorded of the test. When a replicable elastic mechanical behavior is achieved, the strain found in the loading part of the cycle is analyzed using non-contact video strain measurements. By testing the single fiber mechanical properties of these blends as well as the bulk mechanical properties of a fibrous scaffold we are able to determine the mechanical differences of the fibers on both a micro and macro scaffold. These results can be used to better develop tissue engineered scaffolds for blood vessel replacements.

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Structural Stability of Liposomes Modulated by Divalent Cations and an Amyloid

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Liposomes, owing to their unique biocompatibility and biosimilarity, are increasingly used as drug delivery systems and biomembrane models. Divalent cations and proteins have been found to affect the physical-chemical properties of lipid bilayers, but the exact molecular mechanisms and the implications of such interactions, particularly in the case of liposomes, are largely unknown. In the present work we investigated the effect of divalent cations and amyloid on surface-adsorbed liposomes.

Extruded DPPC vesicles with an average diameter of 110 nm were sedimented onto mica surface, and imaged with non-contact-mode atomic force microscopy in aqueous buffer. Preparing the vesicles in buffer containing 0.2, 0.4,